UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

December 20, 2016

MEMORANDUM

Subject: Efficacy Review for Virocid,
EPA Reg. No. 71355-1;
DP Barcode: 435762

From: Sophie Nguyen
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

To: Eric Miederhoff RM31/Tara Flint
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Cid Lines N.V.
Waterpoortstraat 2
8900 Ieper, Belgium

Formulation from the Label:

Active ingredient:	% by Weight
Alkyl dimethyl benzyl ammonium chlorides (50% C_{14},40% C_{12}, 10% C_{16})	17.060%
Didecyl dimethyl ammonium chloride	7.800%
Glutaraldehyde	10.725%
Other ingredients:	64.415%
Total	100.0%

I. BACKGROUND

The product, Virocid (EPA Reg. No. 71355-1), is an EPA registered product designed as a concentrated liquid broad spectrum disinfectant (bactericide, fungicide, and virucide) on hard, non-porous surfaces. The applicant is submitting Data Amendment for the Virocid. The amendment includes changes in the dilution rate for efficacy and to address the product's PDCI issues, confirming effectiveness of the product against *S. enterica*, *S. aureus*, and *T. mentagrophytes*. According to the cover letter, "This supportive efficacy, completed at Accuratus Labs, is additionally being supplied in response to the DCI issued for this registration. Accordingly, a root MRID was requested in advance of this submission to facilitate the subsequent DCI submission. The DCI will include only the MRID number assigned and not the actual data to assure that Antimicrobial Division (AD) does the data review and that duplicate reviews are not performed by the DCI Team". The studies were conducted at Accuratus Lab Services, 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121.

The data package contained a letter to EPA (dated August 26, 2016), EPA form 8570-1 (Application for Pesticide Registration), EPA form 8570-34 (Certification with Respect to Citation Data), EPA Form 8570-35 (Data Matrix), EPA form 8570-27 (Formulator's Exemption Statement), 2 efficacy studies (MRID Nos. 49986301 and 49986302), and the proposed product label. Statement of No Data Confidentiality Claims, Good Laboratory Practice Statement, and Quality Assurance Unit Summary were included with each study.

II. USE DIRECTIONS

The product, Virocid, is designed as a liquid disinfectant for use in farm, animal, and poultry housing facilities and equipment, including hatcheries, food processing plants, and trucks and other vehicles. The label also indicates that the product can be used for fogging hatchery rooms, incubators and hatchers, poultry houses and livestock building. The product can also be used to control of algae and slime forming bacteria in recirculating water cooling systems and evaporative condensers.

Directions to apply product are as followed:

1. **Farm equipment and animal housing buildings (poultry & turkey grow-out houses, laying houses, swine production and housing, barns and large animal buildings):**

For disinfection of hard, non-porous surfaces: stainless, galvanized and painted steel, copper, aluminum, finished wood, vinyl, plastics, glazed tiles, sealed brick walls, aluminum sandwich panels and feeding/drinking equipment:

A. Remove all animals and feed from premises, vehicles and enclosures. Remove all litter and manure from floors, walls and surfaces of barns, pens, stalls, chutes, and other facilities and fixtures occupied or traversed by animals. Empty all troughs, racks, and other feeding and watering appliances.
B. Thoroughly clean all surfaces with soap or detergent and rinse with water. Saturate all surfaces with the appropriate disinfection solution† by using a coarse spray, mop, or sponge. Surfaces must remain wet for 10 minutes.
C. Ventilate buildings and other closed spaces. Do not house animals or employ equipment until treatment has been absorbed or dried.

D. Thoroughly scrub treated feed racks, troughs, and other feeding and water appliances with soap or detergent and rinse with potable water before reuse.
E. Disinfection of equipment: Immerse all halters, ropes, and other types of restraining equipment used in handling and restraining animals, as well as forks, shovels, and scrapers used for removing litter and manure in the appropriate disinfection solution† for 10 minutes. Allow to air dry.
F. Fresh disinfection solution should be made daily or if visibly soiled.

2. Hatcheries:

Remove all animals from the area. Thoroughly clean all surfaces (hatchers, setters, trays, racks, carts, sexing tables, chick boxes, cages) with soap or detergent, then rinse with water. Saturate all surfaces with the appropriate disinfection solution† by using a coarse spray, mop, or sponge. Surfaces must remain wet for 10 minutes. Do not house animals or employ equipment until surfaces have been absorbed or dried. Fresh disinfection solution must be made daily or if visibly soiled.

3. Food processing plants (including Chicken Processing Facilities):

Before using this product, all food products and packaging materials must be removed from the room or carefully protected. Thoroughly clean all surfaces with soap or detergent, then rinse with water. Disinfect hard, non-porous surfaces by applying the appropriate disinfection solution† with a coarse spray, mop, or sponge. All surfaces must remain thoroughly wet for 10 minutes. Allow to air dry. A potable water rinse is required for all surfaces that come into contact with food.

4. Trucks and other vehicles:

Clean all vehicles including mats, crates, cabs, and wheels with high pressure water. Use the appropriate disinfection solution† to treat all vehicles. Leave all treated surfaces exposed to disinfectant solution wet for 10 minutes. Allow to air dry.

Preparation table:

Dilution	Preparation Method
1:170	3/4 fluid ounce per gallon of water
1:200	2/3 fluid ounce per gallon of water
1:256	1/2 fluid ounce per gallon of water
1:400	1/3 fluid ounce per gallon of water

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces (Against a Broad Spectrum of Bacteria):

The effectiveness of disinfectants for use on hard surfaces must be substantiated by data derived using the AOAC Use-Dilution Method (UDM) (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested against each of the three batches of the product at the lower concentration limit (LCL). For UDM, the mean log density for *S. enterica* (ATCC 10708) is to be at least 5.0 and not above 6.0. A mean log density of at <5.0 or >6.0 invalidates the test. The mean log density for *S. aureus* (ATCC 6538) is to be at least 6 and not above 7.0. A mean log density <6.0 or >7.0 invalidates the test. For AOAC Germicidal Spray Products as Disinfectant Method, the mean log density for *S. enterica* (ATCC 10708) is to be at least 4.0 and not above 5.0. A mean log density of <4.0 or >5.5 invalidates the test. The mean log density for *S. aureus* (ATCC 6538) is to be at least 5.0 and not above 6.5. A mean log density <5.0 or >6.5 invalidates the test. To support products labeled as "general disinfectants", killing on 59 out of 60 carriers is required for germicidal spray testing is required. For UDM, conduct three independent tests (i.e., three batches at the LCL tested

on three different test days) against the test microbe. The performance standard for *S. aureus* (ATCC 6538) is 0-3 positive carriers out of sixty. The performance standard for *S. enterica* (ATCC 10708) is 0-1 positive carriers out of sixty. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results for both UDM and Germicidal Spray Products as Disinfectant Method. To be deemed an effective product, the product must pass all tests for both microbes. All products should meet the performance standard associated with the method and microbe at ≤ 10 minutes of contact. The above Agency standards are presented in OCSPP 810.2200.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi):
Efficacy testing should be conducted against *Trichophyton mentagrophytes* (ATCC 9533). Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches at LCL, using AOAC Fungicidal Test. The test should be conducted at 5, 10, and 15 minute exposure times. The inoculum employed should provide a concentration of $\geq 5 \times 10^6$ conidia/mL. **Performance requirements for this test:** the highest dilution that kills all fungal spores is the minimum effective concentration.

Alternatively, the AOAC Use Dilution Method, modified to conform to appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Product Test must be employed. Ten carriers for each of two samples representing two different batches of the product should be evaluated. The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia/carrier. **Performance requirements:** for AOAC International Fungicidal Activity of Disinfectant test, all fungal spores at 10 and 15 minutes should be killed. For the AOAC Use-Dilution Methods, all fungal spores on all 10 carriers should be killed in ≤ten minutes.

Supplemental Claims:
An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same tolerance level.

IV. SYNOPSIS OF SUBMITTED EFFICACY STUDY

According to the Certificate of Analysis that were submitted with the studies for Reg. No. 71355-1, the tested concentrations for **Lot No. S514701** was **21.275% Quaternary Ammonium** and **9.358% Glutaraldehyde**, for **Lot No. S514702** was **21.249% Quaternary Ammonium** and **9.381% Glutaraldehyde**, and for **Lot No. S514703** was **21.319% Quaternary Ammonium** and **9.281% Glutaraldehyde.** The product's nominal concentrations are 24.86% Quaternary Ammonium (17.06% Alkyl Dimethyl Benzyl Ammonium Chloride and 7.8% Didecyl Dimethyl Ammonium Chloride) and 10.725% Glutaraldehyde, and the Lower Certified Limits of the active ingredients are 16.207% Alkyl Dimethyl Benzyl Ammonium Chloride and 7.41% Didecyl Dimethyl Ammonium Chloride (**23.617% Quaternary Ammonium**) and **10.189% Glutaraldehyde**.

1. **MRID 49986301 "AOAC Use-Dilution Method Test Organisms: *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538)" for Virocid (EPA Reg. No.**

71355-1), by Carrie K. Bauer, Study conducted at Accuratus Lab Services, Study completion date – June 27, 2016. Project Number: A20669.

This study was conducted against *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). Three batches (Nos. S514701, S514702, and S514703) of the product, Virocid, were tested using a modified protocol No. SRC46121515.UD.1 (copy provided). On each test date, each batch of the product was diluted further using 1:170 dilutions defined as 1-part test substance + 169 parts 400 ppm AOAC Synthetic Hard Water. Lot# 514701 was tested against *S. aureus* on 4/19/16 and 5/10/16, Lot# S514702 was tested against *S. aureus* on 3/20/16 and Lot# S515703 was tested against *S. aureus* on 4/21/16. Testing against *S. enterica* was performed on 4/19/16 with all three lots. A 10 µL aliquot of the thawed stock was added to a tube containing 10 mL tube of Synthetic Broth. The initial culture was incubated for 24±2 hours at 35-37°C. A 10µL aliquot of the culture was transferred to Morton Closure tubes containing 10 mL of culture medium. Various daily transfers were performed for different test dates, and the final test cultures were incubated for 48-54 hours at 35-37°C. The cultures mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes. The upper portion of the culture was then removed, leaving any clumps and pooled in a sterile vessel and mixed. The *S. enterica* culture was diluted by combining 8.0 mL of test organism suspension with 192.0 mL of sterile growth medium. For testing performed on 5/10/16, the *S. aureus* culture was diluted by combining 35.0 mL of test organism suspension with 35.0 mL of sterile growth medium. The cultures did not include an organic soil load. Sixty (60) stainless steel penicylinder carriers per product batch immersed for 15 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. A maximum of 100 carriers were inoculated per vessel and each vessel inoculated was considered a part of one total inoculum run per test organism. The inoculated carriers were transferred to sterile Petri dishes matted with filter paper. No more than 12 carriers were placed in each dish. The carriers were dried for 38-39 minutes at 35-37°C and at 49.9-52.4% relative humidity, and were used within 2 hours of drying. Each carrier was then placed into a separate tube containing 10.0 mL of the test substance at its use-dilution. For testing performed on 4/19/16, 4/20/16, and 4/21/16, the carriers were exposed for 9.5 minutes at 19.5-20.5°C. For testing performed on 5/10/16, the carriers were exposed for 9 minutes and 45 seconds at 19.5°C. Following the exposure time, the carriers were transferred to 10 mL of neutralizing subcultured medium of Letheen broth with 0.14% Lecithin, 1.0% Tween 80, and 1.0% Glycine. All subcultures were incubated for 48±2 hours at 35-37°C. The subcultures were visually examined for the presence or absence of growth. On 4/21/16, representative test and positive control subculture tubes showing growth were subcultured to Tryptic Soy Agar + 5% Sheep's blood and incubated at 35-37°C for one day. The growth was visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included those of purity, sterility, viability, neutralizer confirmation, and carrier population. The reported average CFU/carrier for ***Salmonella enterica*** **(ATCC 10708)** was **5.21 Log_{10}** and for ***Staphylococcus aureus*** **(ATCC 6538)** was **6.63 Log_{10}.**

Note:

Protocol Amendments: Per Sponsor's request, additional testing will be added to this protocol. Lot S514701 will also be tested against Staphylococcus aureus at a 9 minute 45 second exposure time.

Protocol Deviations: No protocol deviations occurred.

Test History: Testing on April 19, 2016 resulted in failed efficacy results for Lot# 5514701 against *Staphylococcus aureus* following a 9.5 minute exposure time. All data from April 19, 2016 are valid and presented in the body of the report.

Per Sponsor's request, the protocol was amended to conduct additional testing of Lot#S514701 against *Staphylococcus aureus* with an increased exposure time of 9 minutes 45 seconds (see Protocol Amendment 1). Testing of Lot# S514701 against *Staphylococcus aureus* performed on May 10,2016 is considered valid and presented in the body of the report.

2. **MRID 49986302 "AOAC Fungicidal Activity Method Organisms: *Trichophyton mentagrophytes* (ATCC 9533)" for Virocid (EPA Reg. No. 71355-1), by Jamie Herzan, Study conducted at Accuratus Lab Services, Study completion date – July 5, 2016. Project Number: A21009.**

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two batches (Nos. S514701 and S514702) of the product, Virocid, were tested using a modified protocol No. SRC46121515.FACT.2 (copy provided). Each batch of the product was diluted further using 1:400 dilutions defined as 1-part test substance + 399 parts 400 ppm AOAC Synthetic Hard Water. Forty agar plates were inoculated with the stock culture of the organism and were incubated at for 10 days at 35-37°C. The mycelia were removed from all plates and transferred to a glass bottle containing beads and saline/Triton Solution (0.85% saline + 0.05% Triton X-100) and mixed thoroughly. The culture was filtered through sterile gauze to remove hyphal fragments. The conidial concentration was estimated by counting in a hemacytometer. The suspension was standardized to achieve ≥5 x 10^6 conidial/mL by combining 1.0 mL of culture with 3.0 mL of 0.85% saline. The cultures did not include an organic soil load. A volume of 0.50 mL of the test organism suspension was added to a tube containing 5.0 mL of the equilibrated test substance using a sterile pipette. To inoculate the test substance, the tube was removed from the water bath and slanted slightly. The tube was gently agitated and returned to the water bath. Staggered intervals of 1 minute were used. At each exposure time (5, 9.5, and 15 minutes), the tube was removed from the water bath, a sterile 4 mm i.d. loop was inserted into the tube, the sample was withdrawn and transferred to 10 mL of primary neutralizing subculture medium (Sabouraud Dextrose Broth + 0.14% Lecithin + 1.0% Tween 80 + 1.0% Glycine) (Primary and Secondary). The tube was mixed. The test system recovery procedure was repeated for each exposure time from the same tube of inoculated test substance. Secondary subculture transfers were performed by transferring 1 loopful from the primary neutralizing subculture medium to a 10 mL tube of secondary neutralizing subculture medium and the tube was mixed. Subculture tubes were incubated for 10 days at 35-37°C. The subculture plates were incubated for 66-76 hours at 25-30°C for one day. The subcultures were then visually examined for the presence or absence of visible growth. Controls included those of purity, viability, neutralization confirmation, and initial suspension population control. The reported initial suspension population control in CFU/mL for ***Trichophyton mentagrophytes*** **(ATCC 9533)** was **3.2×10^7.**

Note:

Protocol Amendments: No protocol amendment occurred.

Protocol Deviations: No protocol deviations occurred.

V. RESULTS

Hard, Non-Porous Surface Disinfectant – Bactericidal @ 1:170 dilutions

MRID Number	Organism	Contact Time	Lot No.	No. Carriers Exhibiting Growth/Total Carriers	Carrier Population (Average CFU/Carrier)
49986301	*Staphylococcus aureus* (ATCC 6538)	9.5 minutes	S514701 **(4/19/16)**	**4/60**	6.63 $\log_{10}$
		9 min + 45 sec	S514701 (5/10/16)	0/60	
		9.5 minutes	S514702 (4/20/16)	0/60	
		9.5 minutes	S514703 (4/21/16)	0/60	
	Salmonella enterica (ATCC 10708) (4/19/16)	9.5 minutes	S514701	0/60	5.21 $\log_{10}$
			S514702	1/60	
			S514703	1/60	

Hard, Non-Porous Surface Disinfectant – Fungicidal @ 1:400 dilutions

Organism	MRID Number	Contact time	No. Carriers Exhibiting Growth/Total Carriers		Initial Suspension Population (CFU/mL)
			Batch # S514701	**Batch #** S514702	
Trichophyton mentagrophytes (ATCC 9533)	49986302	5 minutes	1°=0/10 2°=0/10	1°=0/10 2°=0/10	3.2×10^7
		9.5 minutes	1°=0/10 2°=0/10	1°=0/10 2°=0/10	
		15 minutes	1°=0/10 2°=0/10	1°=0/10 2°=0/10	

VI. CONCLUSION

1. The submitted efficacy data **support** the use of the product, Virocid (EPA Reg. No. 71355-1), as a disinfectant when the batches were diluted using 1:170 dilutions (in 400 ppm AOAC Hard Water) with bactericidal activity for the following contact time in the absence of 5% organic soil load on hard, non-porous surfaces against the following microorganisms:

MRID #	Organism	Contact Time
49986301	*Staphylococcus aureus* (ATCC 6538)	9 minutes and 45 seconds
	Salmonella enterica (ATCC 10708)	9 minutes and 30 seconds

Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted efficacy data **support** the use of the product, Virocid (EPA Reg. No. 71355-1), as a disinfectant when the batches were diluted using 1:400 dilutions (in 400 ppm AOAC Hard Water) with fungicidal activity 5, 9.5, and 15-minute contact times in the absence of 5% organic soil load on hard, non-porous surfaces against the following microorganisms:

MRID #	Organism
49986302	*Trichophyton mentagrophytes* (ATCC 9533)

Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth.

VII. LABEL RECOMMENDATIONS (for proposed label dated 8/26/16)

Note to PM: The current review of the product only addresses the PDCI issues concerning claims of base microorganisms (i.e., *Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708)) and *Trichophyton mentagrophytes* (ATCC 9533), whose data were submitted with the current data package (DP #435762). Review does not address the PDCI issues concerning additional bacteria, fungus, and virus claims on the proposed revised label.

1. The proposed label claims are acceptable regarding the use of the product, Virocid (EPA Reg. No. 71355-1), as a broad-spectrum disinfectant when diluted using 1:170 dilutions for a 10-minute contact time for use on pre-cleaned hard non-porous surfaces against the following organisms:

   Staphylococcus aureus (ATCC 6538) and *Salmonella enterica* (ATCC 10708)

   These claims **are supported** by the applicant's data.

2. The proposed label claims are acceptable regarding the use of the product, Virocid (EPA Reg. No. 71355-1), as a broad-spectrum disinfectant when diluted using 1:400 dilutions for a 10-minute contact time for use on pre-cleaned hard non-porous surfaces against the following organisms:

   Trichophyton mentagrophytes (ATCC 9533)

   These claims **are supported** by the applicant's data.

3. On the proposed label, registrant must remove the use-directions for “Fogging hatchery rooms, incubators and hatchers, poultry houses and livestock buildings”. Fogging data must be generated to substantiate this application.
4. On page 2 of the proposed label, registrant must remove the word sanitization from “Foam cleaning prior to sanitization or disinfection” and remove any texts that indicate sanitization/sanitizer/sanitize throughout the label.

5. On page 3 of the proposed label, under “Disinfection of non-food surfaces, farm, animal, and poultry housing facilities and equipment”, registrant must revise use-direction number 1. E. to read “Disinfection of equipment: Immerse all previously cleaned halters, ropes, and other types of restraining equipment used in handling and restraining animals, as well as forks, shovels, and scrapers used for removing litter and manure in the appropriate disinfection solution† for 10 minutes. Allow to air dry.”